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INTERNATIONAL BULLETIN
OF PLANT PROTECTION

1945 Nos. 5 & 6

COMPARATIVE EXPERIMENTS ON THE TOXICITY OF DIFFERENT METALS ON *ALTERNARIA, TENUIS* TYPE, AND *PLASMOPARA VITICOLA* *

The investigation of the fungicidal power of compounds or complexes by means of the McCALLAN technique (which we have extensively checked and employed), the bases and developments of which were described in preceding notes, offers a rapid, accurate and practical method of study, provided that all the 'standardization' conditions of the method itself are realized and making the reservation we have already formulated.

For a long time we have been engaged in ascertaining what were the relations existing between a specific toxicity, observed 'in vitro' on *Alternaria, tenuis* type, now commonly used in Italy, and the toxicity which would be manifested in regard to downy mildew of the vine (*Plasmopara viticola*) in the case of the fungicidal compound being dusted on the plants in the open field, that is to say, employed 'in vivo'. In order to be able to reply to the question "how does the toxicity of a fungicidal product compare in regard to the two aforesaid fungi", we undertook experiments the results of which are here summarized.

Although it may seem strange, data of this type were unknown, save a remark of BORZINI (1941) who declares, after the observations he had made, that *Plasmopara viticola* would be approximately 7 to 8 times more susceptible to copper than *Alternaria tenuis*, but he does not indicate the limits of variation of this determination and does not specify the conditions of the validity of this factor. Neither does he extend his observations to metals other than copper.

After some preliminary work on *Plasmopara*, experiments were carried out with a salt for each of the metals tried. Although greater use was made of the metals which could more easily replace copper, because of their availability and

* Communication from Professors R. CIFERRI and E. BALDACCI, Botanical Institute of the University and Italian Cryptogamic Laboratory, Pavia, Italy.

cost (zinc, mercury), if naturally they proved effective, the more precious metals (cadmium, silver) were also studied. We started from the principle that their higher cost would eventually be compensated by greater dilutions, on the understanding that their fungicidal power proves sufficiently high.

Since it was a question of soluble metal salts, the experiments were carried out with the recognized method of the pendant drop on slides in small Ranvier chambers, with the suspension density of the *Alternaria* conidia obtained from mature cultures, after the incubation period, and also at the temperatures previously indicated. Likewise, readings were made and toxicity curves traced following the same rules. Since the germination of the conidia in water (control) has always been very high (from 97 to 99 per cent.), it was considered useless to correct the datum obtained. Each of the data of the toxicity curve represents the average of 10 readings of 100 conidia each (1000 per concentration of each salt) for 5 different experiments with as many sub-cultures of *Alternaria*, with a view to obtaining a truly significant average datum.

In order to obtain an idea of the question, a preliminary determination (a reading of 100 conidia for each concentration) was made on a few concentrations of a salt of numerous metals, even precious or rare, on *Alternaria* conidia, consequently, reserving the execution of more complete experiments to some metals of greater interest as already mentioned.

Preliminary experiments.

In the preparatory tests, with progressive strong dilutions, determination was made of the top and lower limits within which the salt acts in inhibiting the germination of *Alternaria* conidia to the extent of approximately 50 per cent. (LD50, LD being lethal dose).

Without dwelling greatly on these preliminary data which are of a guiding character, we give the results obtained, expressed in gram-atom dilution so as to have comparable data (the figures in brackets indicate the weight of the salt):

Copper sulphate (3.82 gm.): between 1 : 20 and 1 : 50.
Zinc sulphate (2.74 gm.): below 1 : 1.
Nickel sulphate (4.79 gm.): between 1 : 1 and 1 : 5.
Cobalt chloride (4.04 gm.): between 1 : 1 and 1 : 5.
Aluminium sulphate (12.3 gm.): less than 1 : 1.
Gold chloride (1.9 gm.): less than 1 : 1.
Silver nitrate (1.57 gm.): between 1.20 and 1.50.
Barium chloride (1.64 gm.): less than 1 : 1.
Cadmium chloride (1.95 gm.): between 1 : 1 and 1 : 5.
Ammonium molybdate (1.90 gm.): less than 1 : 1.
Uranium acetate (1.7 gm.): less than 1 : 1.
Mercury bichloride (1.35 gm.): between 1 : 10 and 1 : 20.
Ferrous sulphate (4.97 gm.): less than 2 : 1.
Ferric chloride (2.9 gm.): less than 2 : 1.

In excluding the other metals (because of their poor efficacy), there would remain the four most active : copper, mercury, cadmium and nickel. Silver had also to be rejected for although it is practically as active as copper, normally it costs much more than the latter, and it would not be advantageous to use it even recognizing that it has the necessary qualities for practical purposes. Zinc was included despite its low toxicity as it is employed to a fair extent in Italy as a fungicide.

Copper.

In our experiments the toxicity curve for *Alternaria, tenuis* type, of was determined by the reading of 100 conidia per dilution, and efficacy against *Plasmopara viticola* by noting at what concentration the germination of approximately 50 per cent. of the sporangia (LD50) was obtained, independently of the mobility of the zoospores.

Alternaria, tenuis type, inhibition of the germination capacity of the conidia) :

100	40	20	10	7.5	5	2.5	2
96	72	49	30	21	14	3	2 p. p. m.

Plasmopara viticola (idem) :

LD50 = 1–2 p. p. m. (approximately).

Mercury.

Alternaria, tenuis type (as above) :

5.0	2.5	1.0	0.5	0.25	0.1
98	87	62	28	12	1 p. p. m.

Plasmopara viticola (idem) :

LD50 = 1–2 p. p. m. (approximately).

Cadmium.

Alternaria, tenuis type (as above) :

10	7.5	5	2.5	1	0.5
92	82	71	33	13	2 p. p. m.

Plasmopara viticola (idem) :

LD50 = 1–2 p. p. m. (approximately).

Nickel.

Alternaria, tenuis type (as above) :

100	50	10	5.0	2.5	1,0	0,5
97	86	63	51	29	16	7 p. p. m.

Plasmopara viticola (idem) :

LD50 = 2–5 p. p. m. (approximately).

Zinc.

Alternaria, tenuis type (as above) :

1600	400	200	100	40	20	10
92	76	61	30	17	7	3 p. p. m.

Plasmopara viticola (idem) :

LD50 = 50–100 : 1,000,000 (approximately).

DISCUSSION OF RESULTS AND CONCLUSIONS.

(1) The data obtained on the toxicity of the different metal salts on *Alternaria, tenuis* type, have made it possible to trace this scale of toxicity of the different metals tried, starting with hydrosoluble salts and for an equal weight of the metal (gram-atom) : —

Toxicity: Cu = Ag > Hg > Cd = Co = Ni > Au = Ba = Mo = U = Zn > Fe = Al.

(2) The determinations (approximate) of LD50 for *Plasmopara viticola* give the following scale of toxicity for some of the elements tested : —

Toxicity : Cu = Hg = Cd > Ni > Zn

(3) The determinations, by means of the toxicity curve, of LD50 and LD95 for *Alternaria, tenuis* type, gave the following concentrations : —

LD50

Hg	Cd	Ni	Cu	Zn
0.8	3	5	18	155 p. p. m.

LD95

Hg	Cd	Ni	Cu	Zn
3.6	12	80	100	1400 p. p. m.

Namely, for the two levels, Hg > Cd > Ni > Cu > Zn

(4) Consequently, the ratios between the toxicity 'in vitro' of the salts of the five metals tested for *Alternaria, tenuis* type, and for *Plasmopara viticola* (approximately, these, for the germination of the zoosporangia) are variable, and stand more or less as follows :

Toxicity ratios for *Alternaria* and *Plasmopara* : —

Hg	Cu	Cd	Ni	Zn
2 : 1 to 1 : 1	9 : 1 to 18 : 1	3 : 1 to 1.5 : 1	2.5 : 1 to 1 : 1	3 : 1 to 1.5 : 1 (in general approximately).

The data we obtained do not agree much with the toxicity scale of the different metals registered by the investigators engaged in studying this question, in particular that of CLARK, MCCALLAN and WILCOXON, KARTSUIJKER, and of VENEZIA and RUI. In their turn, however, the data of these investigators differ among themselves. KARTSUIJKER himself, for eight species of fungi, gives five different toxicity scales, and for one sole species, two scales, according to whether it is a question of fungistatic or fungicidal action. The toxicity scale of VENEZIA and RUI also differs, but it was only tried for two concentrations and consequently it is difficult to make comparisons. Moreover, these investigators did not examine the inhibition of the germination capacity at LD50 as we ourselves did.

Since *Alternaria, tenuis* type, has not been studied by others, it was impossible for us to make comparisons.

As general conclusion, it is not possible to indicate universal toxicity data valid for all cations or all species of fungi, the variables which affect this phenomenon being too numerous. If, however, it is wished to express the final data in words and not in figures, it is fairly agreed that the toxicity of Cu, Ag and Hg is very high ; that of Cd, high or appreciable, and that of Zn very poor ; the other elements show practically no toxicity.

In reality, *Alternaria, tenuis* type, while it is susceptible to mercury, cadmium and fairly susceptible to nickel, is relatively resistant to copper. From the practical standpoint, this is an advantage, in the sense that there is a very wide margin of certainty between the ' in vitro ' toxicity experiments on *Alternaria* and the possible ' in vivo ' action on *Plasmopara* in the open field. The choice for the theoretical laboratory studies, on the contrary, is less fortunate, precisely because of this relative resistance to copper.

Account should be taken, therefore, of these different susceptibilities of *Alternaria, tenuis* type, to the different metal salts tried (we propose to continue experiments still further), in order to deduce the presumed ' in vivo ' action against the downy mildew of the vine from mixtures studied ' in vitro ' in the laboratory.

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* Drawn up by GIULIO TRINCHIERI, International Institute of Agriculture.

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